Protocols Specifically Used in Our Project

Solid phase extraction for Tc. Wash the HLB SPE column (SupelTM–Select SPE, SUPELCO) with ddH2O for 2 times. Load 5mL sample on to the HLB column. Use the plunger from a 2mL disposable syringe to exert a positive pressure on to the column to elute the sample solution. Extracted droplets are reloaded on to the column and extracted for the second time. Wash the HLB column with ddH2O for 2 times. Add 1mL DMF to the column, use plunger to elute Tc and collect the droplets by a 1.5mL EP tube.

LC-MS analysis for Tc (Water HPLC). MS is first performed to analyze the samples, and specimens with m/z 455 is identified as Tc. According to this LC is subsequently performed (run time: 12 minutes, seal wash period: 5 minutes, solvent A: MeOH 0.1fa, solvent B: water 0.1fa, pressure limits: 0psi to 4000psi). Then use the mixture of graded solvent A (methanol) and solvent B (ddH2O) to wash C18 column (A/B: 5%/95% at 0min and 2min, 40%/60% at 2.10min, 70%/30% at 8min, 90%/11% at 8.10min and 12min). Tc is expected to have a retention time of 6.72 minutes. Integral area of this peak is calculated to quantify the amount of Tc.

Kinetic analysis of enzymes. Use Kinetics mode of the UV-Vis spectrophotometer (Nano Drop, Thermo Scientific). Set wavelength at 360nm, scan every 2s for 200s.

Protein Separation. Transfer the induced sample into centrifuge bucket, centrifuge (4000RCF) at 4°C for 20~25min. Discard the supernatant, re-suspend the precipitation by 7~8mL Lysis buffer with a vortex. Transfer into a 10ml centrifuge tube. Freeze the sample using nitrogen, thaw, sonication for 20 min (for each round sonication 5s, pause 5s) at 200w until the sample is no longer viscous. Divide the sample into several equal aliquots in 1.5-ml microfuge tubes, centrifuge (13,000 RCF) at 4°C for 15 min, keep the supernatant.

Protein Purification. Wash the column once by water, once by Elution buffer, and for 2 times by Lysis buffer. Transfer the sample into the column, shake until well-mixed, keep one tube of eluate for SDS-PAGE. Keep on ice and hake the column for 1h. Take out the column, wash 1 time by Lysis buffer, 1 time by wash buffer (50mM/L, mixed by Lysis buffer and Elution buffer), finally 1 time by Elution buffer (500Mm/L) and keep the eluate. Purify the sample in a millipore filter and centrifuge (3200RCF) at 4°C for 1h until there are 500uL sample left. Add pure water into the filter, centrifuge for another 30min.

Measurement of growth curve. Pick a single colony or add 10μL of frozen bacteria(?) to 400μL LB liquid medium with corresponding antibiotics and shake at 37˚C for 12-14h. After that, the liquid is diluted using LB medium without IPTG for 10 times. 150 microliters of LB containing IPTG for varies concentration gradients (1000, 250, 62.5, 15.625, 3.90625, 0mM) are added to a 96-well microplate (costar 3599). Only the central 60 wells are used in order to prevent evaporation, and the surrounding 36 wells are filled with 150μL of LB medium to buffer this effect. Add 1.5μL of diluted bacteria medium to the wells, furthering diluting them by 100 times. This should make the value of OD_{600} approximate to 0.100. Turn on the microplate reader and start the program. The program is set as follows: Incubate at

37˚C for 10 hours and shake linearly, during which the value of OD_{600} is measured for 10 minutes' interval. Each experiment is carried out by 3 three times.

SEM. Bacteria expressing toxin genes is collected by centrifuge at 13,000 RCF for one minute. Next, sediments are resuspended in 2.5% glutaraldehyde, fixing at 4˚C for at least four hours. Then, specimens are centrifuged at 13,000 RCF for one minute and are washed by PBS for three times. Suspensions are subsequently treated by graded ethanol alcohol (30, 50, 70, 90, 100%) for 15 minutes in each series. Finally, wash specimens with 100% butyl alcohol for three times. Fixed specimens are further lyophilizated and coated with gold. Last, specimens are examined by a Hitachi SU8010.